Induced Spawning Trials with Captive Macquarie Perch, *Macquaria australasica* (Percichthyidae)

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The range and abundance of the native Macquarie perch (*Macquaria australasica* Cuvier) has been greatly reduced during this century. As part of a conservation program (1978-1990), hormone-induction breeding trials were conducted on captive broodfish held in earthen ponds for 109-539 days. Macquarie perch did not spawn naturally in the ponds and water temperatures exceeding 28°C stressed and killed some broodfish. A total of 98 female broodfish (249-425 mm TL, 425-1600 g wt) were examined; 82 were injected either with individual hormones or various combinations of hormones. The only female induced to spawn was injected with three separate doses of 0.5 mg/kg luteinizing hormone-releasing hormone (LHRH). Approximately 1300 eggs were stripped after 89 hours (24 hours after last injection) and 325 larvae hatched. A majority of the 72 male broodfish injected responded to a range of hormones. These results suggest that female Macquarie perch do not undergo complete ovarian maturation in earthen ponds. Possible reasons why females cannot readily be induced to spawn in captivity, and implications for the future conservation of Macquarie perch as discussed.

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INTRODUCTION

The Macquarie perch, *Macquaria australasica* Cuvier (Percichthyidae), is a freshwater fish native to south eastern Australia that is highly regarded for its angling and edible qualities. Its present distribution is limited to small isolated populations in the upper reaches and tributaries of the Lachlan, Murrumbidgee and Murray River systems (Murray-Darling Drainage Division), and in tributaries of the Hawkesbury, Shoalhaven, and Tuross River systems, and the Yarra, Wannon and Barwon Rivers (South-East Coast Drainage Division) (Cadwallader, 1981). These latter populations were originally considered to be derived from fish translocated from the Murray-Darling Drainage Division (Lake, 1971; Cadwallader, 1981); however Dufty (1986) showed that the fish from these two drainage divisions were morphologically and electrophoretically distinct. Dufty further suggested that they are separate species — similar speciation situations have now been documented for other closely related percichthyids in adjacent drainages (Musyl and Keenan, 1993).

Conservation Programs

Historical data indicate that the distribution and abundance of Macquarie perch have declined dramatically during this century (Cadwallader, 1981). Despite there being no clear reasons for this decline, severe environmental degradation or modification associated with river management works, fishing pressure, and competition from

introduced fish species have all been suggested as contributing factors (Cadwallader, 1981). The Macquarie perch is now classified as restricted (Harris, 1987; Pollard *et al.*, 1990) and a number of management strategies have been implemented to conserve the species (Ingram *et al.*, 1990). Two separate artificial breeding programs have been established. The continuing Victorian government program relies on capturing running-ripe females during their annual spawning run in the wild which, at times, is difficult and unreliable (Gooley and McDonald, 1988). The New South Wales (NSW) government program commenced at the Inland Fisheries Research Station (IFRS), Narrandera, in 1978 and continued until 1990.

Objectives

The aims of the NSW government program were to develop artificial breeding techniques using Macquarie perch held in captivity, and release their progeny into areas where this species had formerly been abundant in order to re-establish breeding populations. The strategies and techniques used in this program were based on those previously found to be successful for two other closely related percichthyids, *Macquaria ambigua* (Richardson) and *Maccullochella peelii* (Mitchell) (Rowland, 1983, 1988).

The objectives of this paper are to: report the results of spawning induction trials carried out as part of the breeding program; suggest strategies or techniques for further investigations; and discuss the implications of current knowledge for the future conservation of *Macquarie australasica*.

Materials and Methods

Broodfish Maintenance

Macquarie perch broodfish were collected from Burrinjuck Dam, Googong Dam and the Molonglo River (Murrumbidgee River system), Wyangala Dam and the Abercrombie River (Lachlan River system), and the Shoalhaven River. Due to the low abundance and restricted distribution of this species, mature broodfish could not be caught in large numbers. Fish were transferred to IFRS where they were placed in earthen ponds (surface area 0.04-0.09 ha; maximum depth 2.5 m). Each pond was stocked with both sexes at densities of 104-333 fish/ha (78-85 kg/ha). The diet of Macquarie perch broodfish comprised naturally-occurring food organisms in the ponds, supplemented with live shrimp (*Paratya australiensis* Kemp and *Macrobrachium australiense* Holthuis) and small yabbies (*Cherax destructor* Clark). Individual broodfish were held in ponds for 109-539 days.

Water temperature in each pond was monitored by positioning a minimum/maximum thermometer approximately 0.2 m from the pond bottom. Daily monitoring of water temperature (to the nearest 0.5°C) commenced in August and continued until each pond was drained.

Spawning Induction

When the maximum bottom water temperature in each pond exceeded 15.0°C broodfish were transferred to the hatchery. Fish were anaesthetized with benzocaine (Sigma) at 100 mg/l, and their lengths and weights were recorded. Oocytes and sperm were sampled and examined using the techniques described by Rowland (1983, 1988). Fish were given an initial injection, intraperitoneally at the base of the pelvic fin, on the day of removal from the pond.

The hormone treatments and dosages administered to female Macquarie perch are presented in Table 1. The various hormone treatments were: human chorionic gonadotropin (HCG) (Pregnyl,® Organon Laboratories Ltd); luteinizing hormone-

releasing hormone (LHRH) (HRF,® Ayerst Laboratories Pty Ltd); the luteinizing hormone-releasing hormone analogues (LHRHa), (Des-Gly)^{10} D-Trp^{6}, Pro^{9} LHRH ethylamide (LHRH-At) and (Des-Gly)^{10} D-Ala^{6}, Pro^{9} LHRH ethylamide (LHRH-Aa) (Peptide Technology Ltd); and Ovaprim (Syndel Laboratories Ltd) which contained salmon gonadotropin releasing hormone analogue (SGnRHa) and Domperidone. Combinations of HCG and carp pituitary gland (CPG) (prepared as described by Rowland, 1983), HCG and LHRH-At, HCG and pimozide (PIM) (Jannsen Pharmaceutica Pty Ltd), and LHRH and PIM were also administered. Combinations of hormones were administered in separate injections, one immediately after the other. Male Macquarie perch were given a single injection of either HCG, LHRH, LHRH-At, LHRH-Aa or Ovaprim.

After injection, 2-4 females and 2-4 males were placed together in 2000 l fibreglass tanks that were covered, maintained at either $18 \pm 1°C$ or $20 \pm 1°C$ and aerated.

To determine if ovulation had occurred, female Macquarie perch were anaesthetized and pressure was applied to the abdomen to determine whether eggs could be stripped from the fish. These examinations commenced 35 hours after the first injection and continued daily for up to two weeks. Fish were returned to earthen ponds after each trial. Some individuals were returned to their original ponds; others were moved to new ponds.

Incubation and Hatching

Eggs were stripped and fertilized using the techniques described by Rowland (1988), then placed into Ewos baskets, Downing egg jars or egg incubation aquaria (Hogan, 1988) for incubation. The development of eggs and larvae was monitored daily. When absorption of the yolk sac was completed, larvae were offered live freshwater zooplankton collected twice daily with a 106 μm net from fertilized earthen ponds.

Results

Broodfish Maintenance

Overall survival of Macquarie perch in the ponds was 83%. There were mortalities in two ponds during January 1979, after water temperatures had exceeded 28°C for at least two weeks. Some fish had haemorrhagic areas on the body. Fish that were swimming in a disorientated manner near the surface of the ponds died within several days of being removed to tanks. Broodfish in these ponds did not contain yolky oocytes when examined during the following spring. Although Macquarie perch broodfish recovered from the ponds during 1987 and 1988 were infested with the parasitic copepod, *Ergasilus intermedius* Kabata, no fish died and the outbreaks were successfully treated with a 24 h bath of trichlorfon (Dipterex,® Bayer Australia Ltd) at 2.0 mg/l (Rowland and Ingram, 1991).

At no time did Macquarie perch spawn naturally in the ponds.

Spawning Induction

Maximum bottom water temperatures in ponds at the time when fish were removed ranged from 15°C to 21°C which occurred between 12th September and 22nd November.

Females

A total of 98 mature female Macquarie perch ranging in size from 249 mm to 425 mm total length (TL) and from 425 g to 1600 g weight (wt) were examined.

TABLE 1

Summary of hormone treatments and changes in oocyte diameter for captive female Macquarie perch

Hormone Treatment (dosage units)	No. Fish Injected	Dosages (hours after last injection)				Mean Increase in Oocyte Diameter (%)
		First	Second	Third	Total	
HCG (i.u./kg)	35	250-10000	0-4000 (24-133)	0-2000 (24)	250-10000	2.0
LHRH* (mg/kg)	10	0.5-1	0.1 (21-48)	0-0.5 (22-24)	1-2	6.0
LHRH-At (μg/kg)	7	10-25	0-25 (41-42)	0	20-50	0.8
LHRH-Aa (μg/kg)	7	50	50 (43-45)	0	100	4.6
Ovaprim (ml/kg)	10	0.13-0.5	0-0.5 (30)	0	0.13-0.75	5.2
HCG & CPG (i.u./kg & mg/kg)	3	100-375 & 2-4	0-100 & 0-2 (73)	0	200-375 & 4	6.7
HCG & LHRH-At (i.u./kg & μg/kg)	3	0 & 10	1000 & 0 (19-20)	0	1000 & 10	3.0
HCG & PIM (i.u./kg & mg/kg)	4	1000 & 0-10	0-1000 & 0-5 (41-48)	0	1000-2000 & 5-10	1.0
LHRH & PIM (mg/kg & mg/kg)	3	1.0 & 5-10	0.1 0 & 0-5 (48)	0	1.0-2.0 & 5-10	3.4

* One female subjected to this treatment produced viable eggs which were subsequently fertilized.

Oocytes cannulated from mature fish prior to injection were spherical in shape with a diameter of 1.2-1.8 mm (mean 1.4 mm), pale yellow to amber in colour and opaque or translucent.

Details of hormone treatments and changes in oocyte diameter are presented in Table 1. A total of 82 female Macquarie perch were injected. Oocytes in a majority of fish sampled after injection became clearer and increased in diameter (Table 1). However, only 13 female Macquarie perch could be stripped and eggs from only one of these fish subsequently hatched. This female (340 mm TL, 593 g wt) had received three injections of 0.5 mg/kg LHRH at 0, 41 and 65 hours, and was stripped after 89 hours (24 hours after the last injection). Ovulated eggs were clear, 1.4-1.6 mm (mean 1.5 mm) in diameter and most had one large oil globule. Fertilized eggs swelled to a diameter of 2.2-2.6 mm (mean 2.5 mm) and remained clear. Hatching commenced 6 days after fertilization and continued for 6 days (incubation temperature 17.5-20.5°C). Larvae were 5.0-6.0 mm TL (mean 5.4 mm) at hatching and commenced feeding 3 days after the completion of hatching. A total of 325 larvae hatched from approximately 1300 eggs. Subsequently, this hormone treatment was applied to other female Macquarie perch without success.

Eggs stripped from other females showed no signs of embryonic development. During the 48 hours after attempted fertilization, many of these eggs increased in diameter to 3.1-4.6 mm and many became misshapen, the yolk material contracted, and they eventually burst.

The oocytes of female Macquarie perch that were not stripped eventually became flaccid and clear, and the amount of blood in the ovarian fluid increased.

Males

Male Macquarie perch ranged in size from 190 mm to 390 mm TL and from 185 g to 1500 g wt. A total of 72 males were injected; 36 with 100-900 i.u./kg HCG, 12 with 0.5-2.0 mg/kg LHRH, seven with 5-20 μg/kg LHRH-At, five with 50 μg/kg LHRH-Aa and 12 with 0.25-0.5 ml/kg Ovaprim. All injected fish produced active motile sperm.

Discussion

Macquarie perch broodfish maintained in earthen ponds during this study could not be induced to spawn using exogenous hormones. The testes of males appeared to develop fully and produce viable sperm, whereas only one female of the 82 treated was successfully induced to spawn. Similarly, female Macquarie perch held in ponds and tanks at the Snobs Creek Freshwater Fisheries Research Station and Hatchery (Victoria) have failed to spawn after hormone injections (G. Gooley, 1992, *pers comm*).

The size of eggs sampled from Macquarie perch in this study suggests that the ovaries of pond-held fish did not complete vitellogenesis and final maturation. Although the cannulated eggs were similar in appearance to those in wild fish described by Gooley and McDonald (1988), ovulated eggs from the single successful spawning obtained during this study were substantially smaller (1.4-1.6 mm diameter) than those ovulated by Macquarie perch captured in Dartmouth Dam (1.75-2.00 mm diameter) (Gooley and McDonald, 1988). Cadwallader and Rogan (1977) reported that the eggs of Macquarie perch spawned in Lake Eildon (Victoria) were 1-2 mm in diameter and increased to 4 mm in diameter within 20-30 minutes after fertilization. In contrast, the fertilized eggs obtained in the current study increased to only 2.2-2.6 mm in diameter. Possible reasons for the incomplete gonadal maturation of female Macquarie perch are discussed below.

Broodfish Maintenance

Unsuitable environmental conditions, including the absence of species-specific cues will inhibit normal gonadal development and spawning in fishes (Donaldson and Hunter, 1983; Bye, 1984). In the wild, Macquarie perch undergo an annual upstream migration during spring and early summer to spawn in flowing water over small boulders and gravel beds (Wharton, 1968; Cadwallader and Rogan, 1977). The absence of these environmental conditions in earthen ponds may have inhibited final ovarian maturation, ovulation and spawning. However, attempts by Wharton (1973) to simulate conditions suitable for spawning, by holding fish in aquaria with gravel substrate and in earthen ponds with flowing water, were also unsuccessful. Other factors which may have influenced ovarian maturation, and ovulation in Macquarie perch held in earthen ponds are: high summer water temperatures, such as those which stressed and killed Macquarie perch in 1979, poor nutrition due to inappropriate diets, unsuitable water quality, and general stress associated with captivity (Billard *et al.*, 1981; Watanabe *et al.*, 1984).

Some variability in gonadal maturation may be part of the normal reproductive biology of Macquarie perch. Gooley and McDonald (1988) found that Macquarie perch participating in the annual spawning migration in Dartmouth Dam showed substantially different stages of oocyte development and that this was reflected in the variable, and unpredictable success of spawning induction using HCG.

Spawning Induction

In general, female fish at an advanced stage of ovarian maturation, usually where vitellogenesis is complete, respond readily to the injection of exogenous hormones (Lam, 1982; Donaldson and Hunter, 1983; Shelton, 1989). Dosages of 500-1000 i.u./kg HCG, which have been used to induce spawning in female Macquarie perch caught during the annual spawning migration in Dartmouth Dam (Gooley, 1986; Gooley and McDonald, 1988), and repeating the hormone treatment that induced the single successful spawning during this study, failed to induce spawning in other captive female Macquarie perch. This failure adds further support to our hypothesis that ovarian maturation in female Macquarie perch held in earthen ponds was inconsistent and generally incomplete.

Future Research and Conservation

The apparent lack of complete gonadal development in female Macquarie perch held in earthen ponds, and the unreliability and difficulty of collecting running-ripe females from the wild (Gooley and McDonald, 1988), have important implications for the conservation of this species. All programs to date have been unsuccessful in rearing large numbers of Macquarie perch for re-introduction into areas within the former distribution. While the species remains threatened, efforts to conserve it should continue. Although NSW Fisheries has terminated its captive breeding program, it is planned to impose a total ban on the capture of Macquarie perch across the state to ease the pressure on remaining stocks. Research into breeding of Macquarie perch is continuing at the Snobs Creek Freshwater Fisheries Research Station and Hatchery (Victoria).

The authors consider that the long-term conservation of this species depends, at least in part, on the development of captive breeding techniques. The 1978-1990 study has identified areas that need urgent investigation before a breeding strategy, for captive Macquarie perch, can be developed; these are listed below.

1. Nutritional requirements of broodfish and the effects of diet on gonadal development.

2. Stimuli required for the later stages of gonadal maturation in broodfish held in earthen ponds.
3. Use of alternative hormones including LHRHa, antiestrogens, corticosteroids, progestogens and prostaglandins, and methods of application, such as sustained-release implantable pellets.

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